

# Protocols for the Basic Crosslinking Experiment

## 1. Prepare the protein solutions

All the proteins were stored in the 20mM TBS (pH=7.3), and the concentration of specific protein was previously detected by Thermo® NANODROP 2000 Spectrophotometer. For experimental use, those solutions were diluted to about 1mg/mL by certain volume of 20mM TBS (pH=7.3). The final concentration of protein solutions are shown below:

Protein Contained	Mw (kDa)	Mass Concentration	Molar Concentration
3A-SUP	21.4	1.018mg/mL	47.59 $\mu$ M
3A-mSA	25.4	1.209mg/mL	47.59 $\mu$ M
3B	55.4	1.000mg/mL	18.05 $\mu$ M

Solutions were made at room temperature (25C°).

## 2. Establish the reaction system

Two experiments same to each other were made to ensure parallelity.

The volume of reactive system is 20 $\mu$ L, and the ratio between monomers containing A and 3B was 5.5:14.5, which is the result of obeying the rule that the quantity of A and B must be approximately equal to each other (For example, the molar ratio between 3A-SUP and 3B in such system is nearly 1:1). particular temperature (25C°) and particular pH (pH=7.3). Reactants were firstly placed on heaters for 5 minutes to ensure they had reached the reactive temperature, and then the reactants were

mixed according to their temperature and the reactions began. The whole process lasted for 2 hours.

### **3. Post-processing**

All the reactive systems were added 17.5 $\mu$ L Protein Loading Buffer (7.5 $\mu$ L 5x protein loading buffer mixed with 10 $\mu$ L TBS). Then samples were immediately added into the tubes and boiled at 95C° for 7 minutes. After the boiling process, samples were placed at 25C° for 10 minutes to ensure every sample were cooled, and then centrifugation was introduced to shake down the condensate water in the purpose of keeping the volume. Then, adding 7.50 $\mu$ L for reactive samples and 5.00 $\mu$ L for ThermoFisher® Protein Markers to the wells and running SDS-PAGE for a certain time at 150V was required, followed by Coomassie Brilliant Blue staining process. After the process, SDS-PAGE gels were scanned and analyzed by software lane 1D to find out the surplus content of monomers (must be with negative control groups) and the mass distribution of oligomers. Results were further analyzed to be charted or graphed.